UNIVERSITI PUTRA MALAYSIA

OPTIMIZATION OF HYDROLYSIS OF EDIBLE BIRD'S NEST AS POTENTIAL PREBIOTIC INGREDIENT

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FSPM 2020 6

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By

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Thesis Submitted to the School of Graduate Studies, Universiti Putra Malaysia, in Fulfilment of the Requirements for the Degree of Master of Science.

July 2020

Abstract of thesis presented to the Senate of Universiti Putra Malaysia in fulfilment of the requirement for the degree of Master of Science

OPTIMIZATION OF HYDROLYSIS OF EDIBLE BIRD'S NEST AS POTENTIAL PREBIOTIC INGREDIENT

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July 2020

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Edible Bird's Nest (EBN) is dried gelatinised salivary secretion from the swiftlets, *Aerodramus fuciphagus* and *Aerodramus maximus* during their breeding season. Edible bird's nest was reported to possess various health benefits and has been widely consumed since the ancient dynasty in China with their practices of Traditional Chinese Medicine (TCM). Scientific evidence supported this statement with the nutritional content of EBN, which consists of unique and high valued glycoprotein, sialic acid, epidermal growth factor (EGF), and other components that promote anti-oxidative, anti-hypertensive, immunity and more. Today, EBN is even utilised in a broad range of industries such as food, medical, pharmaceutical, and cosmetic industries. However, the utilisation and consumption of EBN are bounded due to certain properties such as insolubility. This limitation is overcome by the invention of bioactive EBN hydrolysate which simplifies the glycoprotein into glycopeptide and free peptide with improvement in solubility, functional, and nutritional properties. In this study, cleaned house EBN underwent alcalase enzymatic hydrolysis for 1, 2, 3, and 4 hours to investigate the optimal conditions for the production of EBN hydrolysate. The degree of hydrolysis of EBN suggested that 60 – 90 minutes of alcalase treatment is sufficient to release EBN micro-particles from the inactive core protein. Through hydrolysis, the physicochemical properties of EBN showed that the macro glycoprotein and protein breakdown into glycopeptide and free peptide, while leaving the amino acid, sialic acid, and glycan part remain untouched. Further on as a food ingredient, the consumption of EBN and information regarding the digestibility and prebiotic activity of EBN remain unknown. In this research, the prebiotic potential of EBN and hydrolysate were evaluated through *in vitro* digestion using amylase, pepsin, pancreatin, and bile, followed by *in vitro* fermentation with colon model that mimics the human gastrointestinal environment. Samples obtained at 0, 6, 12, and 24 hours were evaluated for bacterial enumeration by fluorescent *in situ* hybridisation (FISH) and organic acids production by high-performance liquid chromatography (HPLC). The population of *Bifidobacterium* and *Lactobacillus* have demonstrated

a positive prebiotic effect in EBN and hydrolysates similar to the fructooligosaccharides (FOS) that act as a positive control. The raw EBN (ER) and hydrolysate that undergo 1-hour hydrolysis (EH-1) also highlighted a significantly higher inhibition in the growth of pathogenic *Clostridium histolyticum* group when compared to FOS. The metabolites analysed for acetate, propionate, and butyrate showed a production similar to FOS, especially in ER and EH-1 with a regular production trend. This is the contribution of the bioactive glycan-oligosaccharides peptides in EBN that partially resists digestion of which remained intact until reaching the colon and being fermentable for colonic microbiota, leading to the prebiotic potential of EBN itself. Protein and peptide as the major nutrients in EBN were observed to contribute to low production of valerate, isovalerate and isobutyrate especially in ER and EH-1. This phenomenon has precluded the possibilities of toxicity in the colon. The study found that the EBN and hydrolysate are able to enhance colonic bacteria, promote probiotic growth, suppress pathogen and produce beneficial metabolites, therefore able to function as a prebiotic ingredient that promotes colon health.

Abstrak tesis yang dikemukakan kepada Senat Universiti Putra Malaysia sebagai memenuhi keperluan untuk Ijazah Master Sains

PENGOPTIMUMAN HIDROLISIS SARANG BURING WALIT SEBAGAI RAMUAN BERPOTENSI PREBIOTIK

Oleh

TAN HUI YAN

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Sarang burung walit (SBW) adalah rembesan air liur yang telah kering dihasilkan oleh burung walit (*Aerodramus fuciphagus* dan *Aerodramus maximus*) semasa musim pembiakan. Sarang burung walit (SBW) yang dilapor dengan pelbagai faedah kesihatan telah menyebabkan penggunaan SBW yang sinonim dalam amalan perubatan tradisional oleh masyarakat Cina sejak dahulu lagi. Kajian saintifik telah menunjukkan SBW mempunyai glikoprotein yang unik dan bernilai tinggi, faktor pertumbuhan epidermis (EGF), asid sialik dan sebagainya. Nutrien ini telah membawa pelbagai faedah kesihatan seperti antioksidan, antihipertensi, meningkatkan immuniti dan lain-lain lagi. Kini, SBW telah pun diaplikasikan dalam industri-industri seperti makanan, perubatan serta kosmetik. Walaubagaimanapun, aplikasi dan penggunaan SBW dihadkan kerana sifat-sifat seperti ketidaklarutan. Masalah ini telah diatasi dengan penghasilan bioaktif hidrolisat SBW yang menjadikan glikoprotein kepada glikopeptida dan peptida. Ciptaan ini telah menambahbaik ciri-ciri SBW dari segi kelarutan, fungsi dan nutrien. Dalam kajian ini, SBW yang bersih telah dirawat dengan *alcalase* enzim hidrolisis selama 1, 2, 3 dan 4 jam untuk pengeluaran hidrolisat SBW. Kajian daripada darjah hidrolisis SBW mencadangkan bahawa rawatan *alcalase* selama 60 – 90 minit adalah cukup dan cekap untuk mengeluarkan zarah mikro SBW daripada protein terasnya yang tidak aktif. Dengan proses hidrolisis, sifat fizikal-kimia SBW menunjukkan glikoprotein telah dihancurkan kepada glikopeptida dan peptida, tanpa mempengaruhi asid amino, asid sialik dan glikan dalam SBW. Selanjutnya sebagai bahan makanan, informasi tentang SBW dari segi penghadaman dan aktiviti prebiotik masih belum diketahui. Dalam kajian ini, potensi prebiotik SBW dan hidrolisat dinilai berdasarkan kaedah penghadaman *in vitro* dengan enzim amilase, pepsin, pankreatin dan garam hempedu, seterusnya diikuti proses penapaian *in vitro* dengan rekaan model usus besar sebagai pendua keadaan gastrousus manusia. Sampel penapaian yang diperoleh pada 0, 6, 12 dan 24 jam telah melalui penghitungan bakteria dengan analisa *fluorescent in situ hybridization* (FISH) dan analisa asid organik dengan *high performance liquid chromatography* (HPLC). Pertumbuhan *Bifidobacterium*

dan *Lactobacillus* melalui penapaian SBW dan hidrolisat telah menunjukkan kesan prebiotik bahan tersebut seperti mana perbandingan dengan fructooligosakarida (FOS) yang berperanan sebagai kawalan positif. Di samping itu, SBW mentah (ER) dan hidrolisat (EH-1) daripada 1 jam hidrolisis dapat membantutkan pertumbuhan patogen *Clostridium histolyticum*. Penghasilan metabolit seperti asetat, butirat, propionat daripada proses penapaian SBW dan hidrolisat menunjukkan produksi seakan yang dapat dilihat pada penapaian FOS. Kesan positif ini boleh dikaitkan dengan kewujudan glikan-oligosakarida dalam SBW dan hidrolisat. Sifat separa-rintang pencernaan oleh glikoprotein SBW sekali gus menyumbang kepada pertumbuhan probiotik dalam usus besar. SBW yang sebahagian besarnya terdiri daripada protein dan peptida telah menyumbang kepada penghasilan valerat, isobutirat, dan isovalerat dalam kuantiti rendah, terutamanya SBW mentah (ER) dan hidrolisat EH-1. Fenomena ini telah mengelakkan kemungkinan berlakunya toksik di dalam usus. Kesimpulannya, SBW dan hidrolisat (EH-1) menunjukkan potensi sebagai prebiotik bagi menggalakkan pertumbuhan probiotik seterusnya membantu dalam mengekalkan kesihatan usus manusia.

ACKNOWLEDGEMENTS

I would like to thank my parents, Mr Tan Jit Lee and Madam Ng Siew Mee, who always pointed out my ways when I lost direction in life and choices. Their effort in putting my education as their priority made me who I am today. To my lovely Grandmother, Mrs Tan Sye Khim who always told me to be diligent and taught me to be organised in my work in my daily life. She is the reason that I can multitask and enjoy the moment. Gratitude also to the Lui family who loves me and supported me all along, Mr Sebastian Lui, Madam Shelly Lee, and my siblings Selina, Sean and Sachairi Lui. Thank you for their endless support and love in my journey of Master Degree.

This is also a dedication to my late Grandpa, Mr Tan Gee Meng who taught me to embrace challenges with courage and a spirit of never give up. As he always said, "*you are the hope for you own dead end, make your path, turn your despair into hope and light them up for yourself*." I hope you are with me on the whole journey.

Special thanks to my supervisor, Assoc. Prof. Dr Shahrul Razid Sarbini, co-supervisors and lecturers Prof Dr Abdul Salam Babji, Dr Lim Seng Joe and Dr Wong Sie Chuong for their guidance, patience, support, encouragement and generosity in sharing knowledge in teaching and guiding me throughout my research. I would also like to express my deepest appreciation to my teammates, Muhamad Hanif bin Rawi, Siti Maisarah binti Mohd Nashri, Ravisangkar Ramachandran, Hidayati Syamimi binti Mohd Noor, Leanne Mun Sue Lian and Nur' Aliah Daud for their guidance and support. Besides, I would like to thank our laboratory assistants, Miss Georgina Sylvia ak Niwin and Miss Sawiyah Tori for their assistance throughout my research.

Last but not the least, thanks to my friends, Chen Shi Yin, Chin Jing Ni, Chai Lee Ling, Kathleen Michelle, Mayrilyn Solo Thompson, Nur Bazilah Binti Burhan, Nathaniel Bin Mikal, Ali Maru, Muhammad Amirul Hamzah bin Muhd Umar, Hamad Mohamad Salah Hamad Ahallil, my heartfelt thanks to them for always motivating me throughout my studies. My thanks also go to those who supported me in any way during my research.

I certify that a Thesis Examination Committee has met on 13 July 2020 to conduct the final examination of Tan Hui Yan on her thesis entitled "Optimization of Hydrolysis of Edible Bird's Nest as Potential Prebiotic Ingredient" in accordance with the Universities and University Colleges Act 1971 and the Constitution of the Universiti Putra Malaysia [P.U.(A) 106] 15 March 1998. The Committee recommends that the student be awarded the Master of Science.

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This thesis was submitted to the Senate of Universiti Putra Malaysia and has been accepted as fulfilment of the requirement for the degree of Master of Science. The members of the Supervisory Committee were as follows:

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LIST OF ABBREVIATIONS

EBN	Edible Bird's Nest
TCM	Traditional Chinese Medicine
EGF	Epidermal Growth Factor
SCFA	Short Chain Fatty Acid
BCFA	Branched Chain Fatty Acid
GIT	Gastrointestinal Tract
NSP	Non-Starch Polysaccharides
FOS	Fructooligosaccharides
LDL	Low-Density Lipoprotein
AA	Amino Acids
EAA	Essential Amino Acids
PEAA	Partially Essential Amino Acid
NEAA	Non-Essential Amino Acids
ADCC	Antibody-Dependent Cellular Cytotoxicity
CDC	Complement-Dependent Cytotoxicity
HFD	High Fat Diet
ACE	Angiotensin i-Converting Enzyme
ROS	Reactive Oxygen Species
PAS	Periodic Acid/Schiff
MW	Molecular Weight
DH	Degree of Hydrolysis
TSS	Total Solid Solubility
HPLC	High-Performance Liquid Chromatography
SSF	Simulated Salivary Fluid
SGF	Simulated Gastric Fluid
SIF	Simulated Intestinal Fluid
FISH	Fluorescence *in situ* Hybridization
PHS	Phenol Sulphuric Acid
DP	Degree of Polymerisation
LAB	Lactic Acid Bacteria

CHAPTER 1

INTRODUCTION

1.1 Study Background

Edible bird's nest (EBN) is dried gelatinised salivary secretion from swiftlet during the breeding season (Guo et al., 2006). In Malaysia, the common source of consumable EBN is mainly the white and black EBN produced from *Aerodramus fuciphagus* and *Aerodramus maximus* respectively. EBN has been widely used since antiquity, especially in China with their practices of Traditional Chinese Medicine (TCM) far dated in the Tang and Ming Dynasty (Lau & Melville, 1994). In the Chinese community, EBN is also recognized as "*yanwo*", which brings the meaning of bird's nest of swiftlets (L. S. Chua & Zukefli, 2016; Gao, Qiao, & Geng, 1988).

Edible bird's nest has notably value as the "Caviar of the East" due to its medicinal and nutritional beliefs in TCM for treating several health disorders and consumptive diseases such as autoimmune diseases, coughs, tuberculosis, asthma, stomach ulcer, gastrointestinal disorders, promoting physical and mental strength, and anti-ageing thus maintaining youthfulness (L. S. Chua & Zukefli, 2016; Lim et al., 2002; Marcone, 2005). Today, several scientific studies on EBN even provide evidence in supporting the claims of EBN in TCM. For example, the positive evidence on immunity enhancing properties by Ng et al. (1986), the epidermal growth factor (EGF) that promotes anti-ageing effects (Kong et al., 1987), ganglioside and brain structuring by sialic acid (Chan, 2006) and anti-influenza prevention properties by (Guo et al., 2006).

These health benefits of EBN is the contribution of the nutritional component. Research on the nutritional composition of EBN highlighted that EBN consists of 80 – 90 % of unique glycoprotein (Babji, Nurfatin, Etty Syarmila, & Masitah, 2015). This glycoprotein contributes to the substantial amount of protein and carbohydrate at approximately 60% and 25% respectively (Kathan & Weeks, 1969). The rest of the nutrients include minerals such as calcium, potassium, sodium, magnesium, zinc, iron and manganese (Marcone, 2005). Thus, EBN is widely consumed by many people around the world.

Today, various of EBN products such as instant bird's nest in glass bottles are very much commercialised in the market (Babji et al., 2015; Bojsen et al., 2007). The great health values of EBN has also encouraged the application and utilisation in more industries. For instances, EBN tonic soup in the food industry, supplements in the pharmaceutical industry, and skincare ingredient in the cosmetic industry. Commonly, EBN extract is involved in the products instead of the EBN as a whole material. This is because the application of EBN in products is bounded due to the limitation of certain physicochemical properties such as insolubility. This issue is overcome by the invention of bioactive EBN hydrolysate

by Babji et al. (2014) using enzyme technology. In which, alcalase hydrolysis was performed on EBN glycoprotein to produce simpler glycopeptide and free peptides.

This development on EBN was reported to achieve high recovery of more than 90% and ease the application in various products. However, the optimization of hydrolysis condition by means of the nutritional bioavailability and physicochemical properties are lacked in information. Therefore, the study was to hydrolyse EBN for 1 to 4 hours and investigate the physicochemical properties changes with the degree of hydrolysis. Previously, few studies have shown result that hydrolysis of EBN improves the solubility and functionality. For examples, anti-oxidative activity by Nurul Nadia et al. (2017) and anti-hypertensive properties by Nurfatin et al. (2016). However, as a food ingredient, no study was reported regarding the digestibility and prebiotic activity of EBN and never mentioned for EBN hydrolysate.

In this study, an *in vitro* gastrointestinal process on double-boiled raw EBN and EBN hydrolysate of different hydrolysis period was carried out in a colon model. The digestibility of EBN was determined by the quantity loss after *in vitro* digestion. Whereas, the prebiotic potential of EBN and hydrolysates was determined by evaluation of the colonic bacterial population and metabolites production during *in vitro* fermentation.

1.2 Significance of Study

The significance of the study includes:

a. There is scarce information for parameters of optimum hydrolysis on EBN and the physicochemical properties differences of EBN and hydrolysates are unclear. In which, it is crucial for the utilisation of EBN in different industries with specific criteria and requirements.
b. Further on as a food ingredient, it is proposed that enzymatic hydrolysis of EBN may ease the absorption and assimilation of its nutrition in the human gastrointestinal system due to simpler micro-particles. Previous research has reported that the glycan part of EBN glycoprotein is able to enhance solubility and resists proteolytic digestion (M. C.-M. Chung, 1984; Cole & Smith, 1989; Schachter, 1986a; Ralph T. Schwarz & Datema, 1982). However, the digestibility of EBN in the human gastrointestinal system is yet to be studied.
c. The possibility of EBN in resisting the proteolytic digestion thus provide them with the possible strength to remain intact until reaching the colon and lead to the prebiotic potential of EBN itself (M. C.-M. Chung, 1984; Cole & Smith, 1989; Schachter, 1986a; Ralph T. Schwarz & Datema, 1982). Until today, no study was done regarding to the fermentation and prebiotic activity of EBN.

1.3 Objectives

The objectives of this study include:

a. To optimize the hydrolysis of EBN and investigate the physicochemical differences of raw EBN and hydrolysates with different hydrolysis period.
b. To determine the digestibility of raw EBN and hydrolysates.
c. To determine the prebiotic potential of EBN and hydrolysates by evaluating the colonic bacterial population and organic acids production in *in vitro* colon model.

1.4 Hypotheses

The hypotheses of this study include:

a. There is a significant difference in physicochemical properties of EBN at different hydrolysis period.
b. The EBN and hydrolysates showed the ability to resist digestion.
c. The EBN and hydrolysate are able to manipulate the growth of gut microbiota and the production of short chain fatty acids (SCFA).

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